Supplementary information: A ruthenium-oligonucleotide bioconjugated photosensitizing aptamer for cancer cell specific photodynamic therapy

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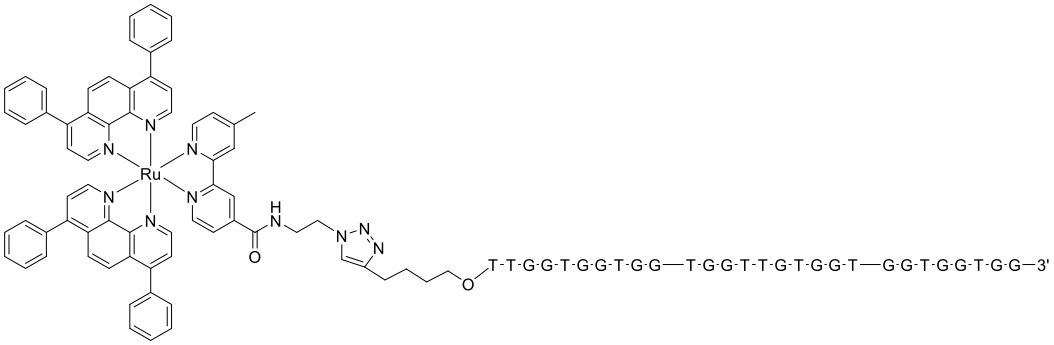
Figure S22. ^{1}H NMR spectrum of **bpyN$_3$**

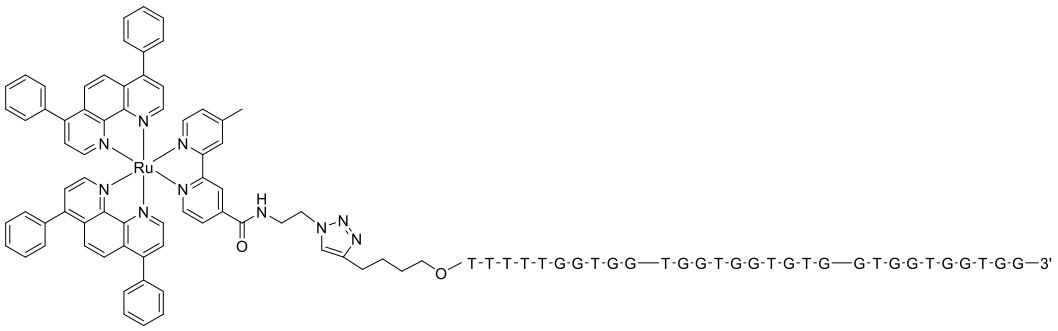
Figure S23. HR-MS ESI spectrum of **bpyN$_3$**

Figure S1.Chemical structures of **AS1411-5’-TT-Ru**, **AS1411-5’-TTTTT-Ru** and **AS1411-3’-TTT-Ru**

AS1411-5’-TT-Ru



AS1411-5’-TTTTT-Ru



AS1411-3’-TTT-Ru

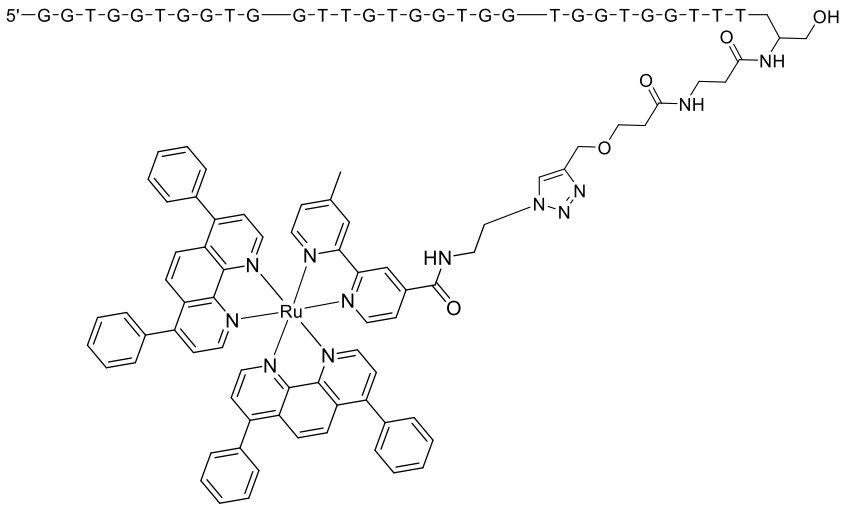
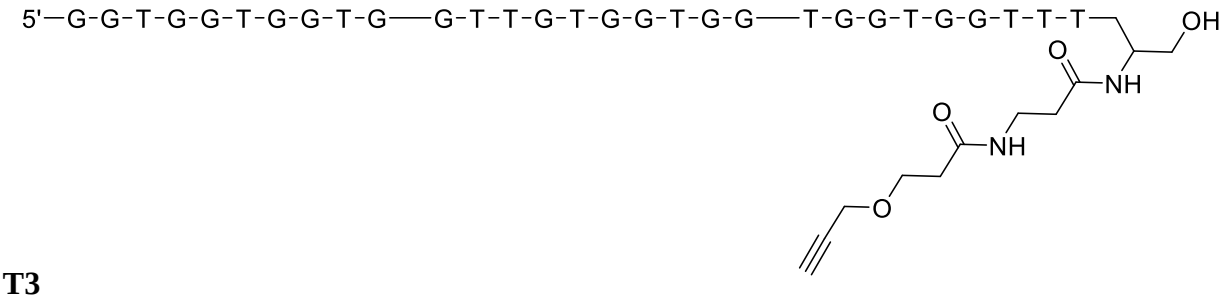
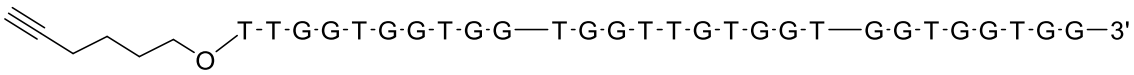


Figure S2. Sequences with alkyne linker chemical structures for **T2**, **T3** and **T4**;

T2



T3



T4

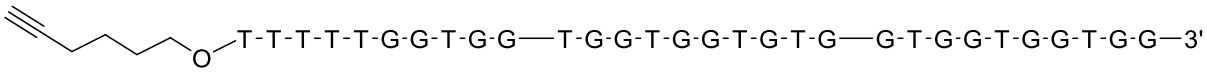


Figure S3. ^{1}H NMR spectrum of **RuN_3** (400 MHz, in CD_3CN)

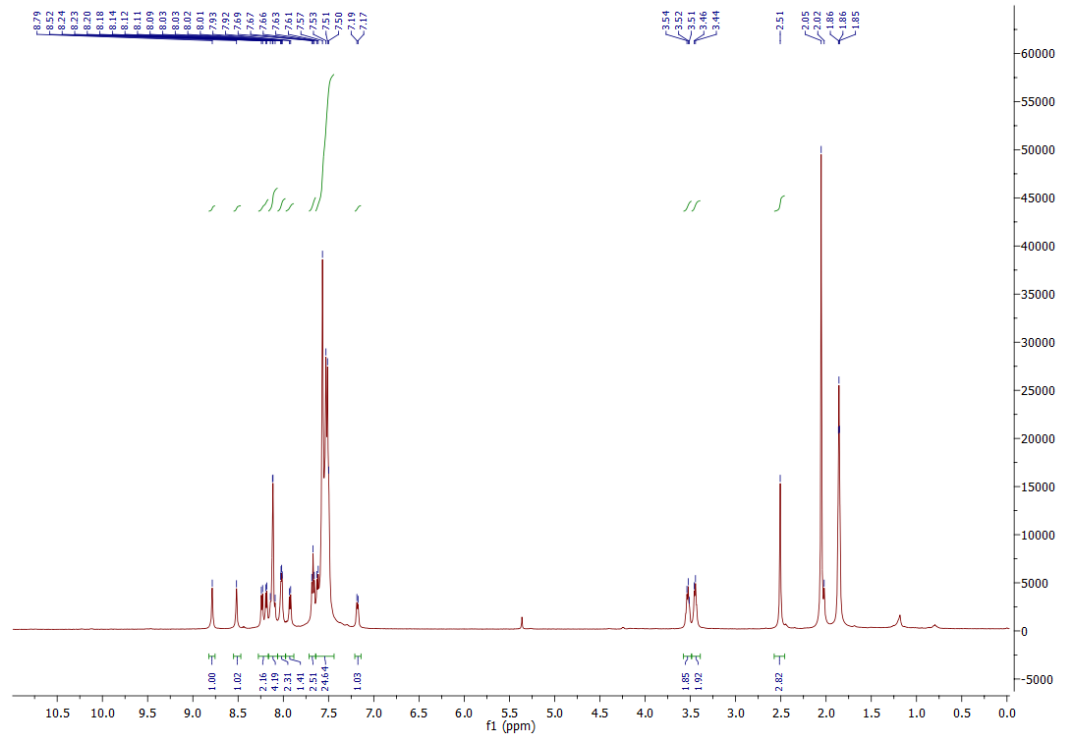


Figure S4. ^{13}C NMR spectrum of **RuN_3** (101 MHz, in CD_3CN)

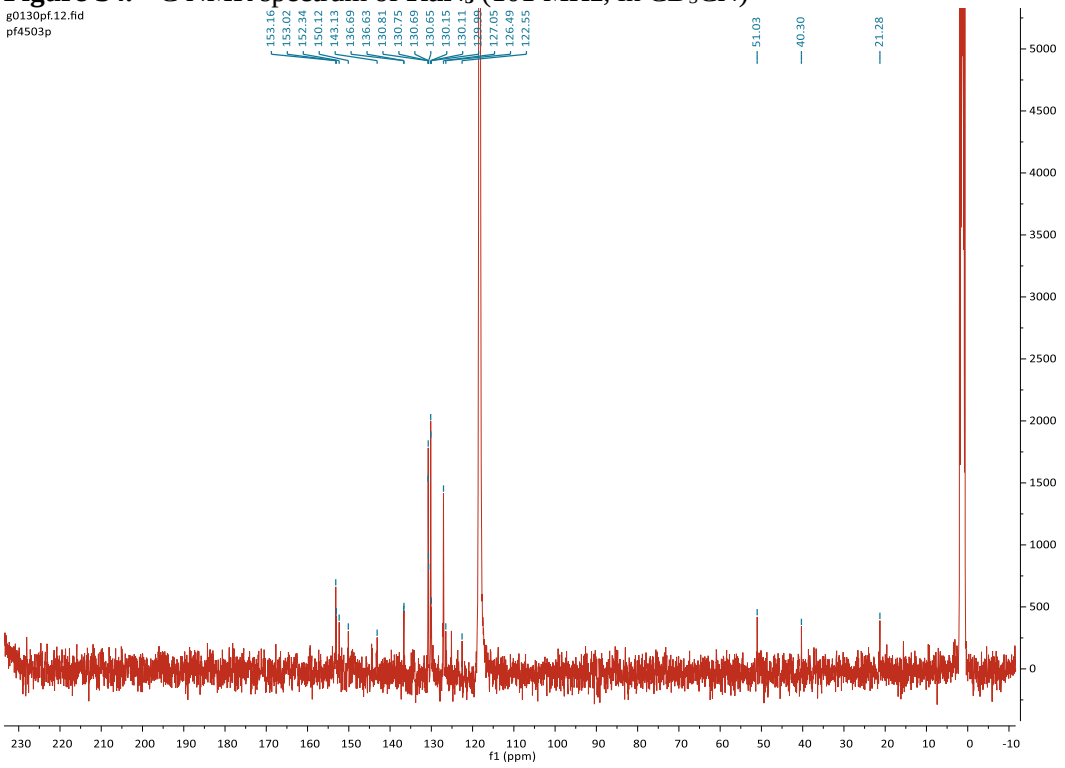


Figure S5. DEPT-135 NMR spectrum of **RuN_3** (101 MHz, in CD_3CN)

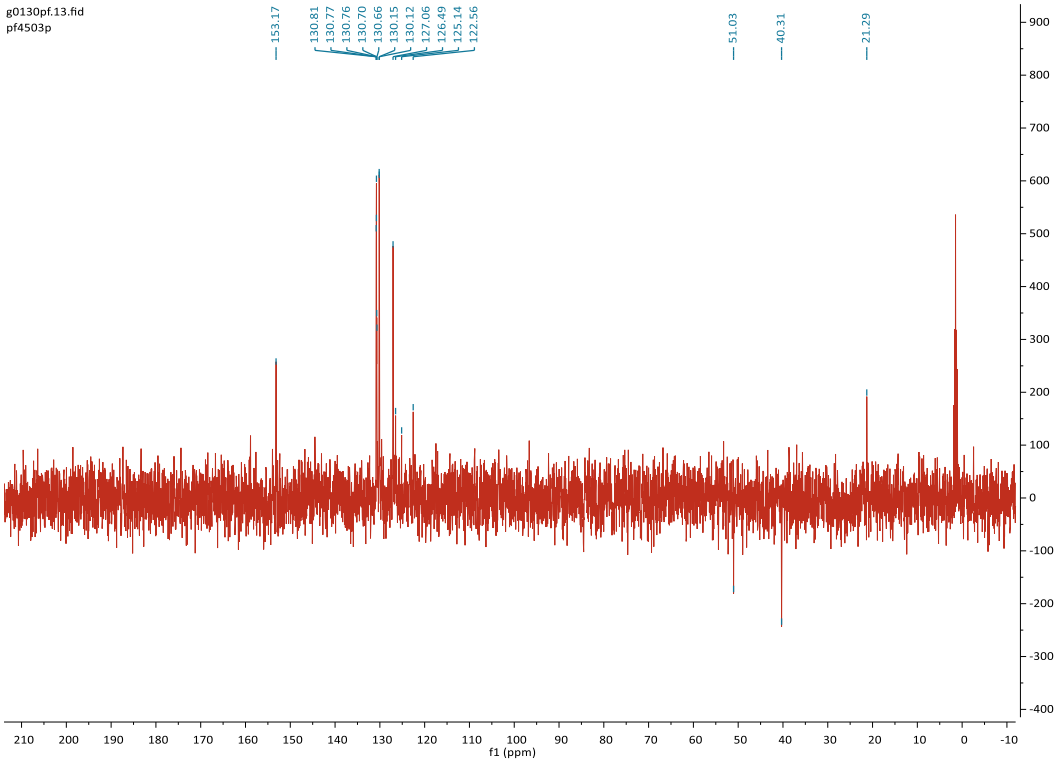


Figure S6. ^{19}F NMR spectrum of **RuN_3** (367 MHz, in CD_3CN)

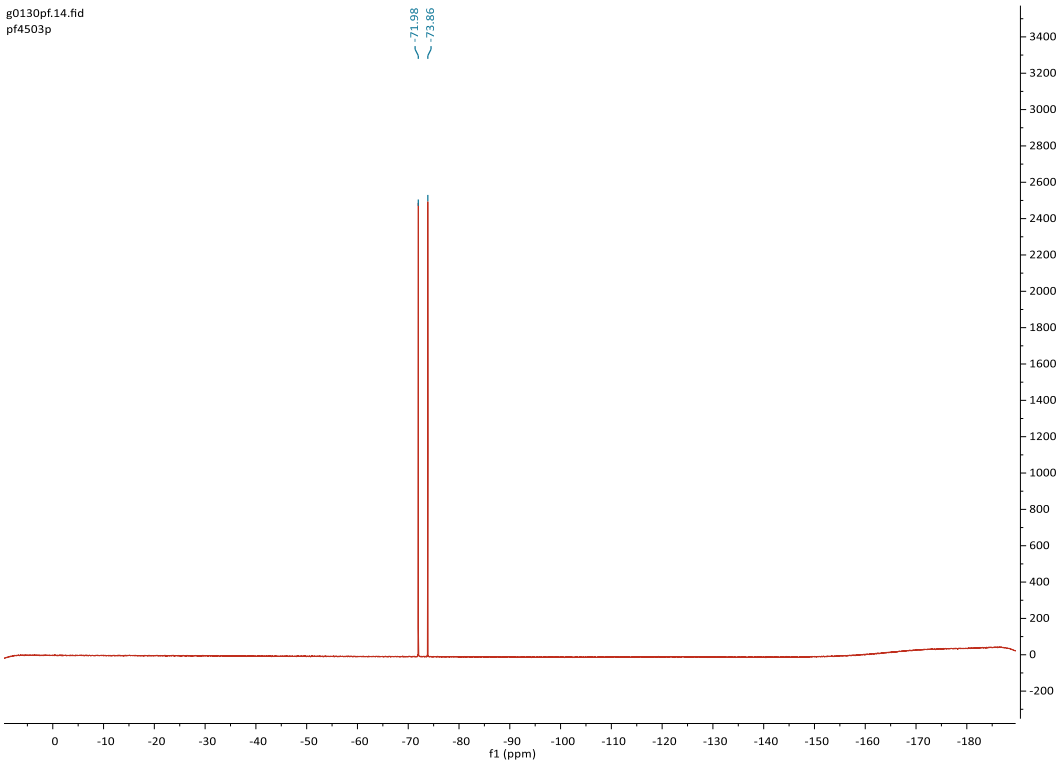


Figure S7. HSQC spectrum of **RuN_3** (400 MHz, in CD_3CN)

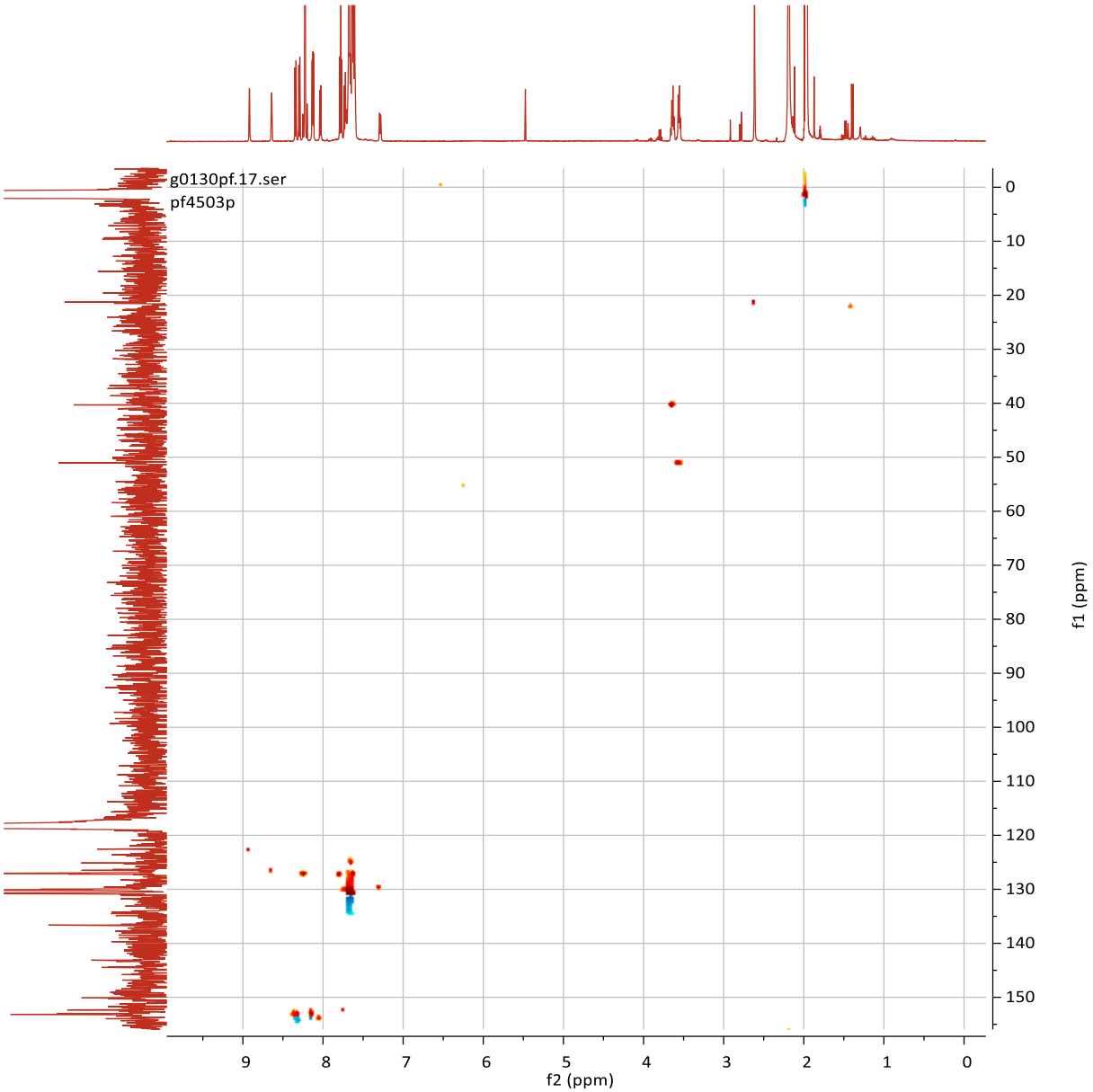


Figure S8. COSY spectrum of **RuN_3** (400 MHz, in CD_3CN)

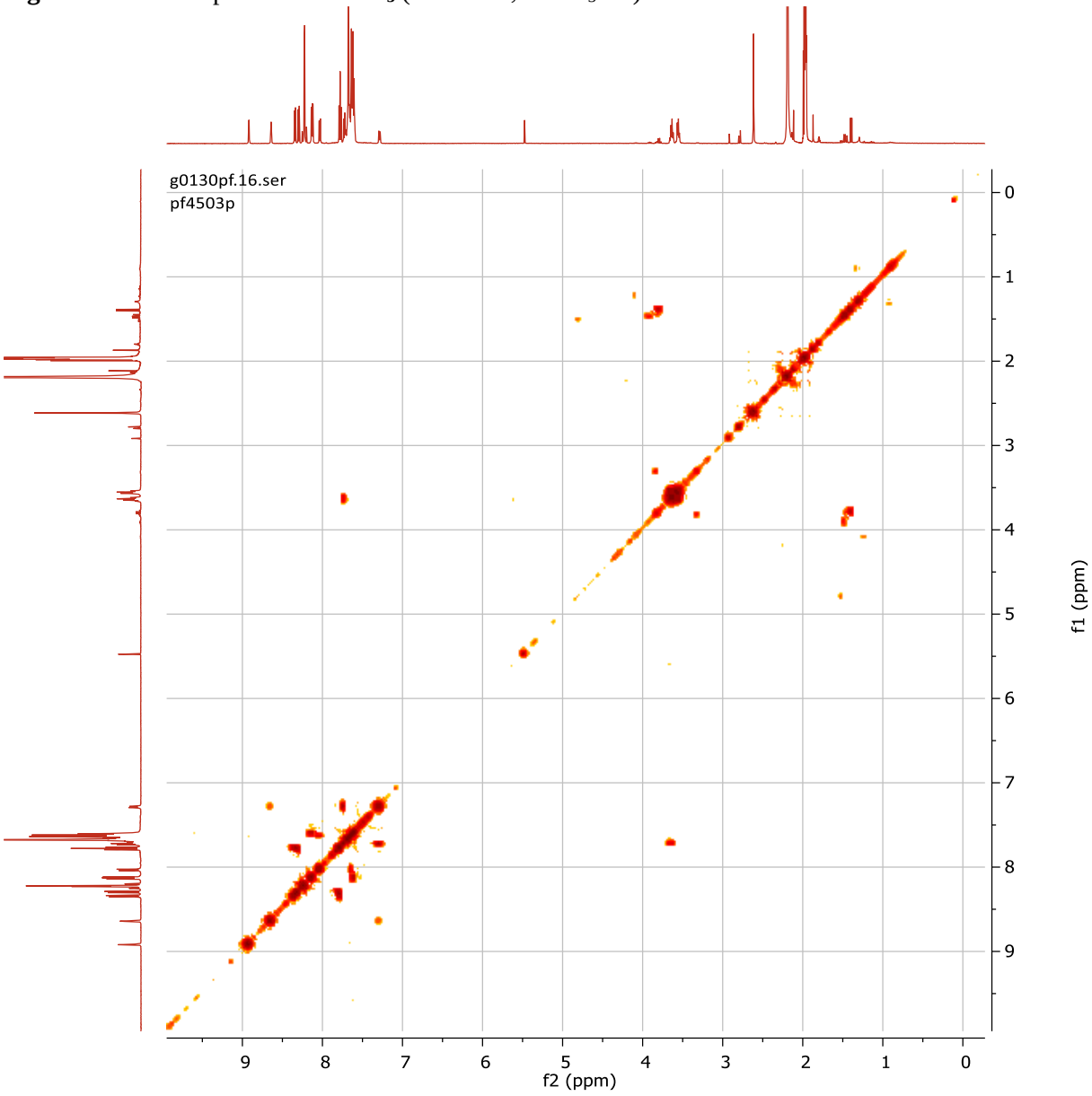
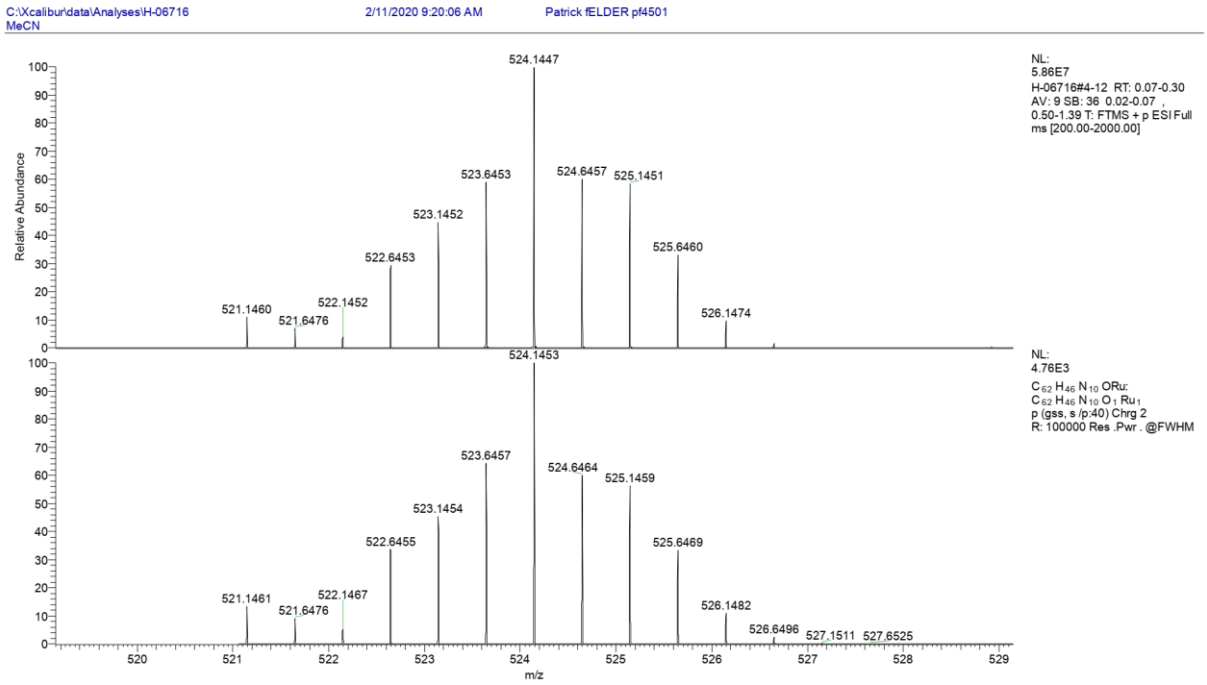


Figure S9. HR-MS ESI spectrum (positive mode) of **RuN$_3$** (in MeCN)



Error = 0.0 ppm; Relative Intensity (%) 100

Calculation of monoisotopic masses – 1.00728 Th (-H^+).
–18.0338 Th (-$NH4^+$).
–22.98922 Th (-Na^+).
–38.96316 Th (-K^+).

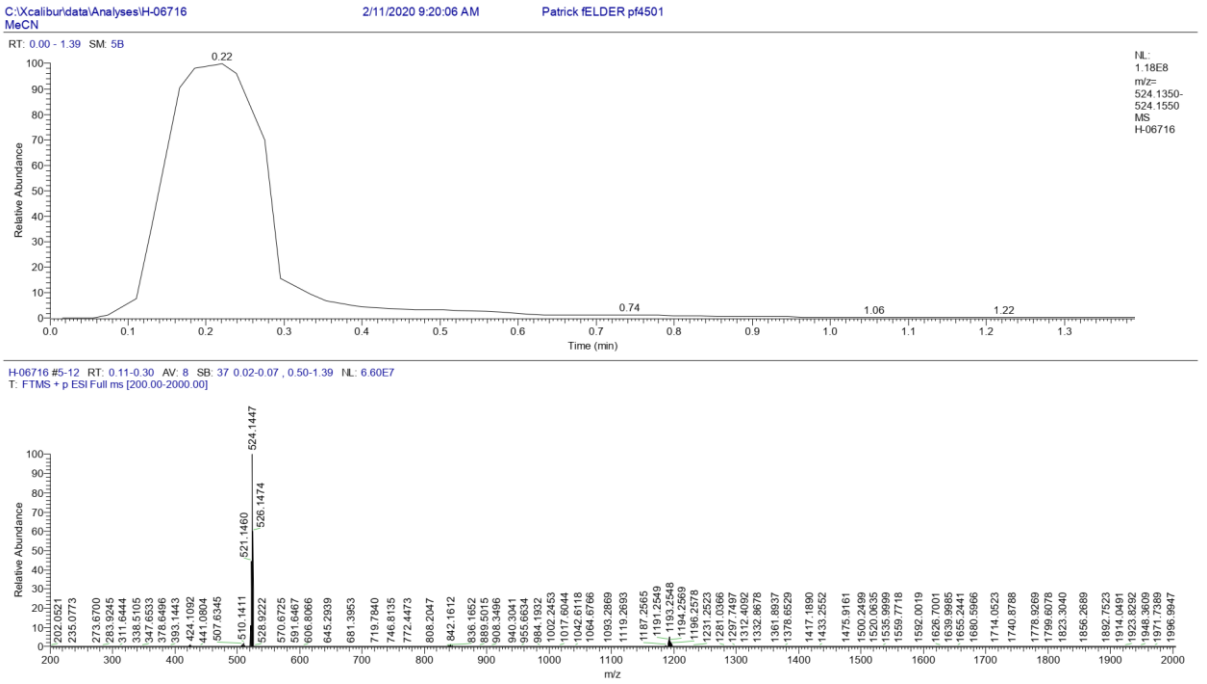


Figure S10. IR spectrum of **RuN_3**

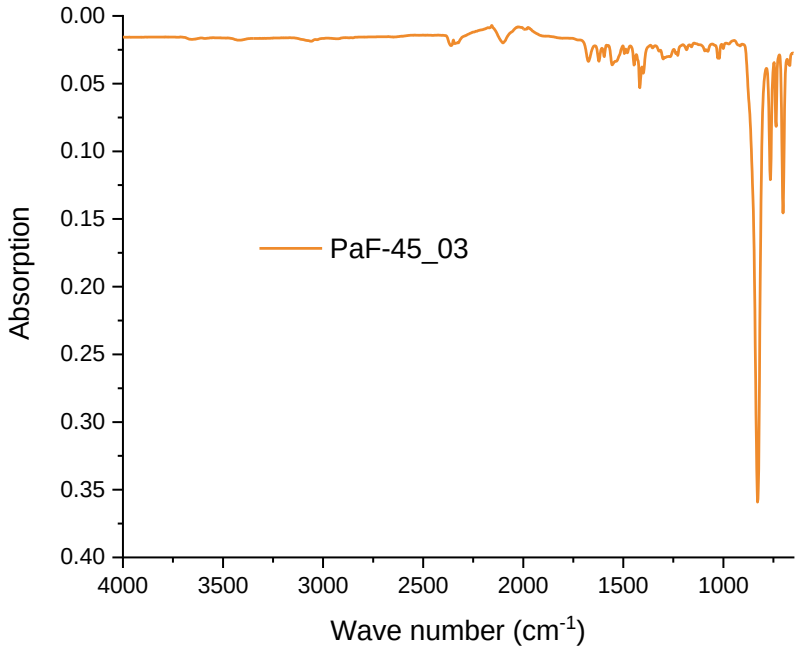


Figure S11. a) Absorption spectrum of **RuN$_3$** in CH_3CN; b) Emission spectrum of **RuN$_3$** in CH_3CN

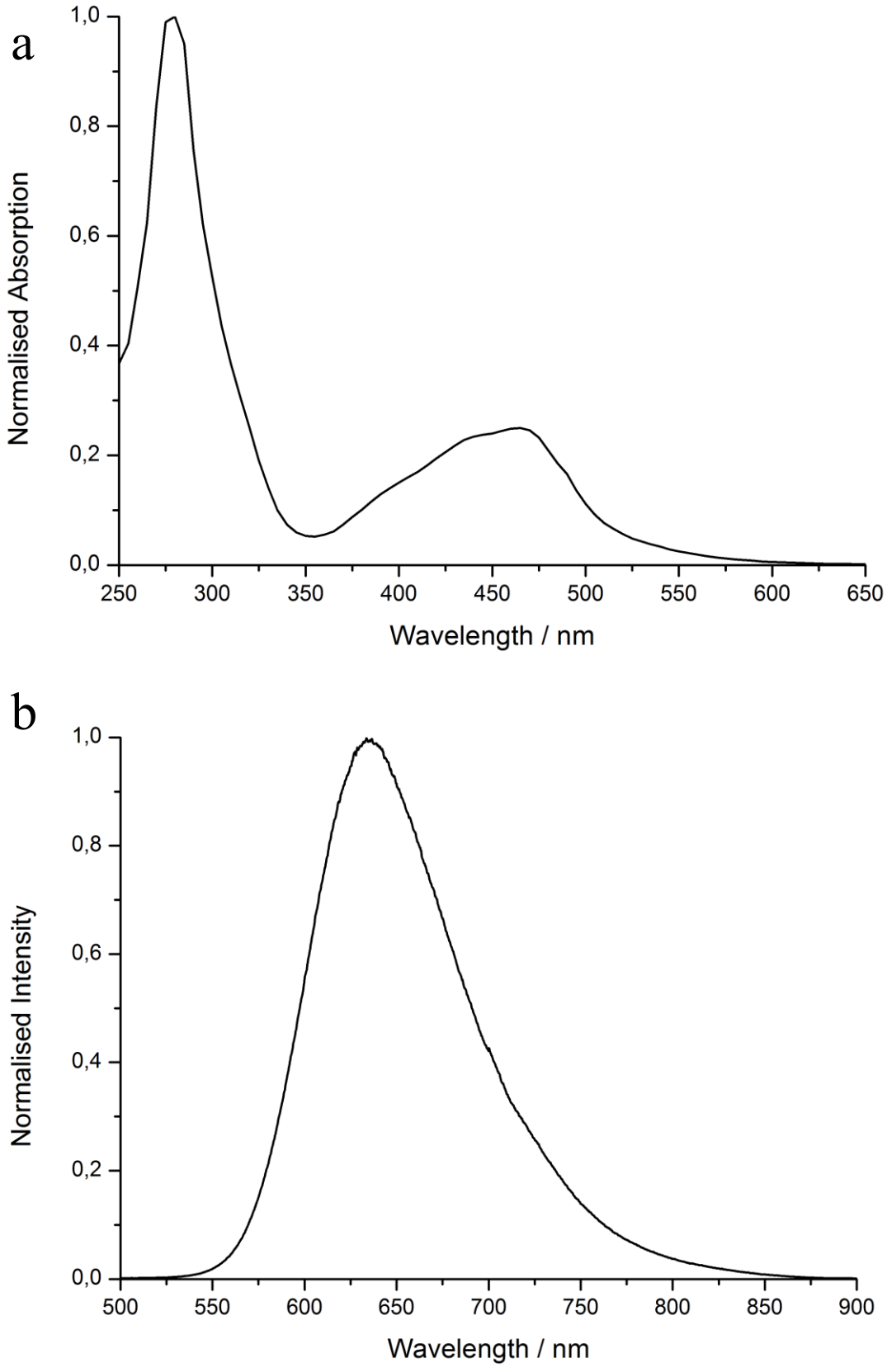


Figure S12. PAGE gel of a) - **T5** and b) - **AS1411-3'-TTT-Ru** (1st batch – original click conditions)

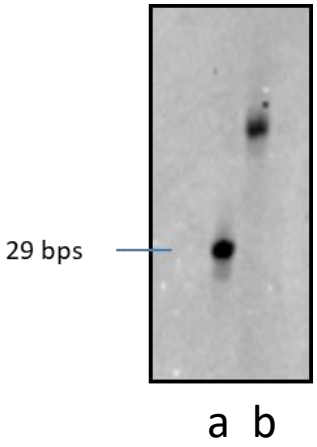


Figure S13. CD spectra of a) **AS1411-3'-TTT-Ru** (1st batch – original click conditions) and b) **AS1411** (**T1**) with increasing KCl concentration.

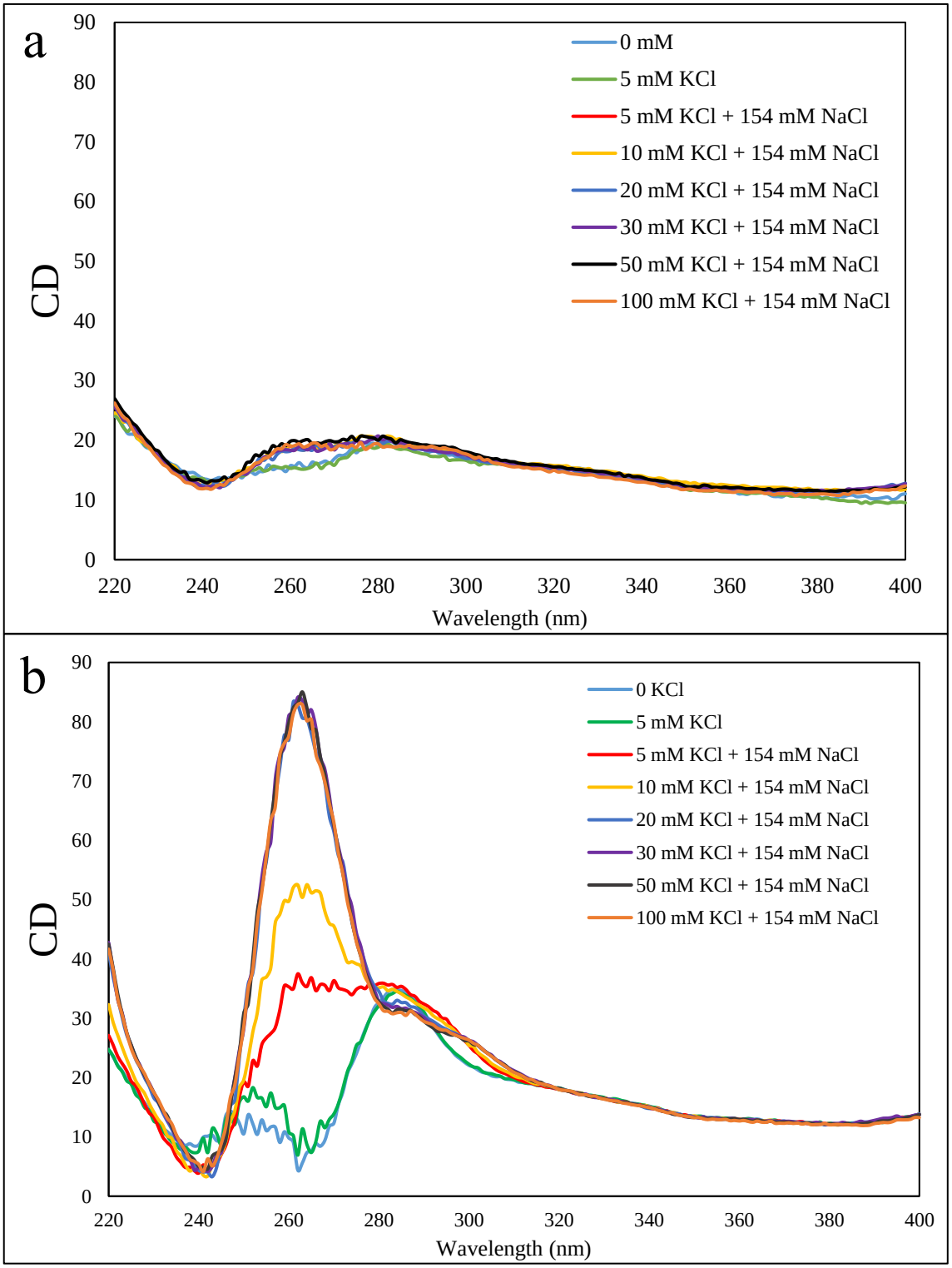


Figure S14. Cell surface nucleolin staining using anti-nucleolin antibody staining (ZN004, magenta) with Nucblue costaining (cyan) in HT29 cells (cell surface nucleolin expressing cell line) and RPE-1. λ_{exc} = 405 nm (NucBlue) λ_{exc} = 488 nm (Anti-Nucleolin). Scale bar = 20 microns

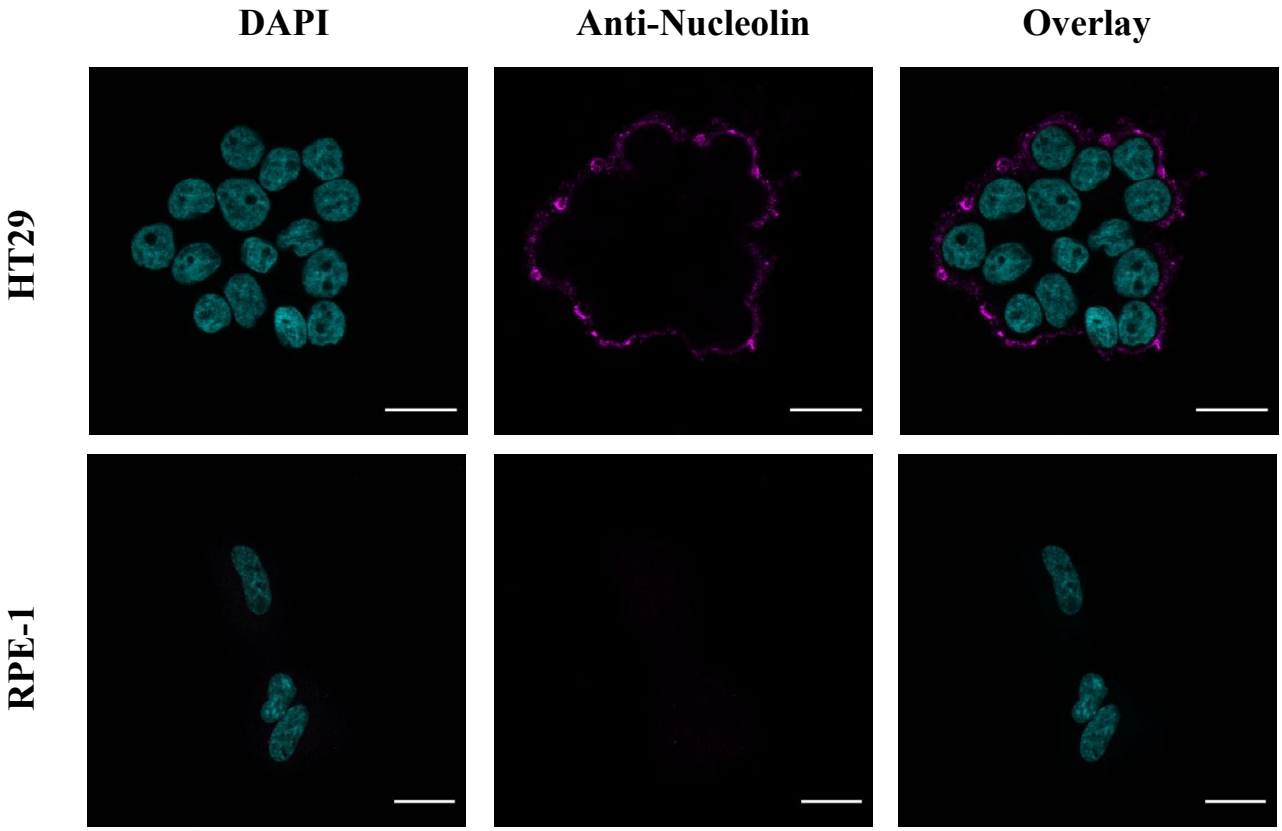
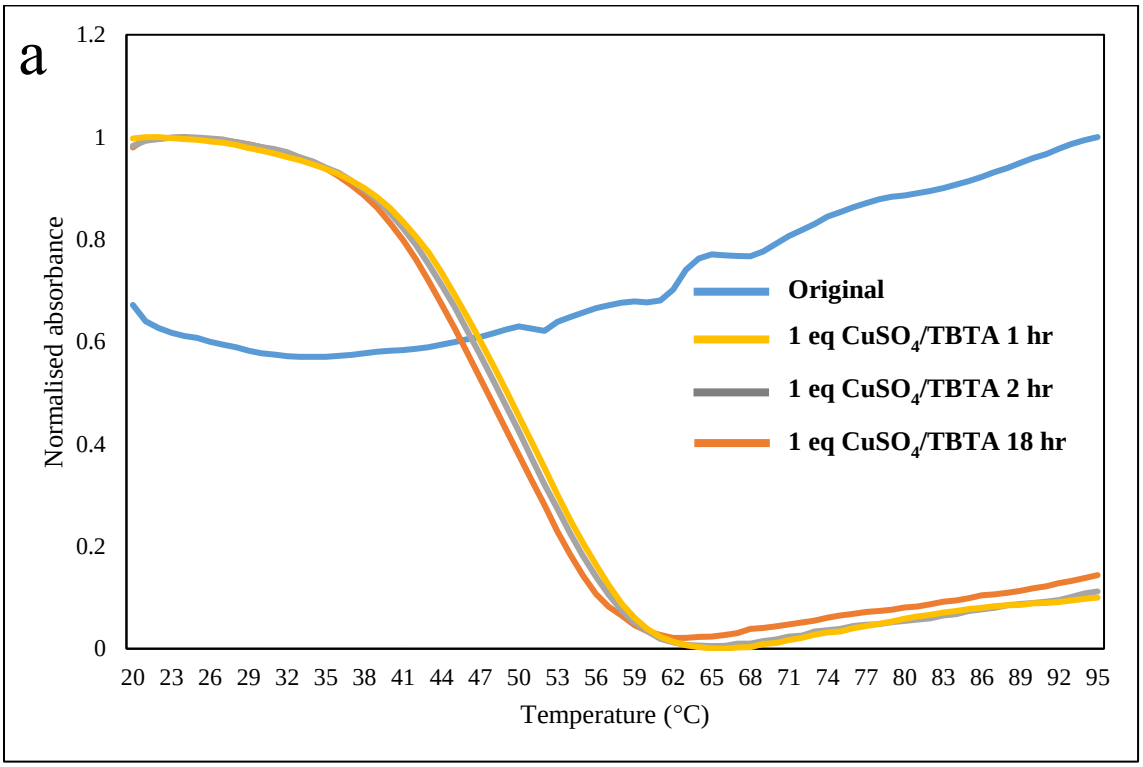


Figure S15. Tm melting curves for a) **T2** after exposure to various click reaction conditions; b) **AS1411, AS1411-5'-TT-Ru, AS1411-5'-TTTTT-Ru** and **AS1411-3'-TTT-Ru**



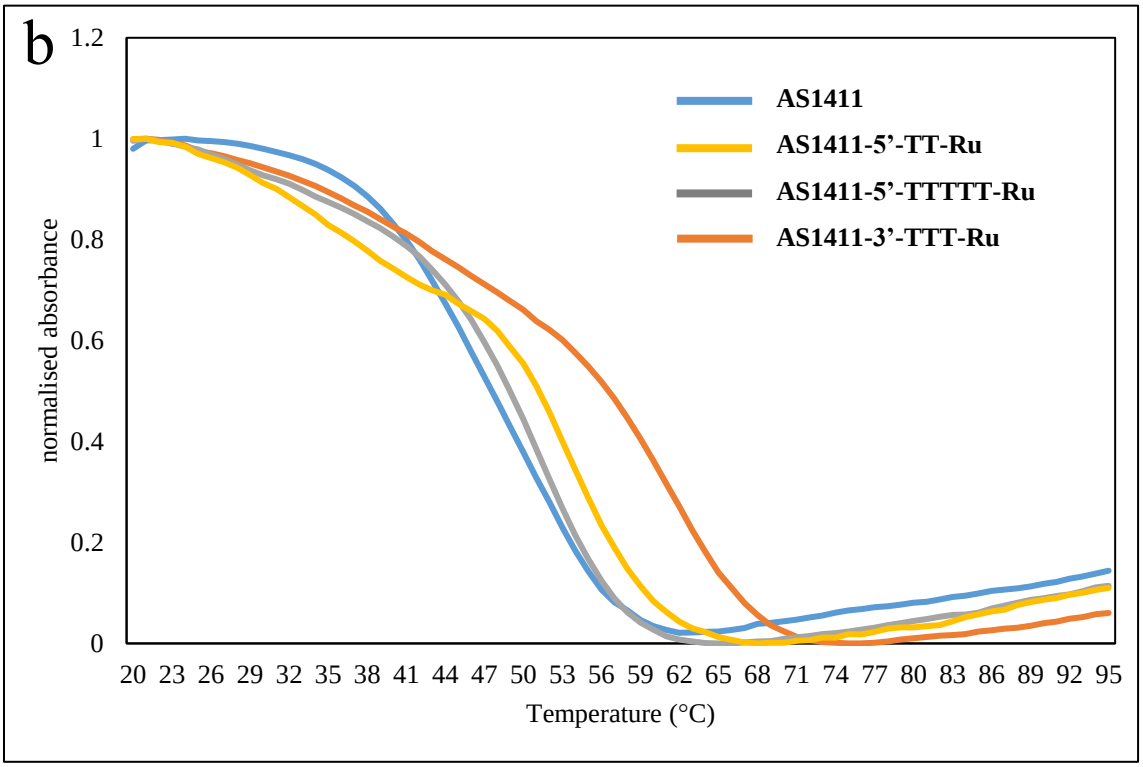
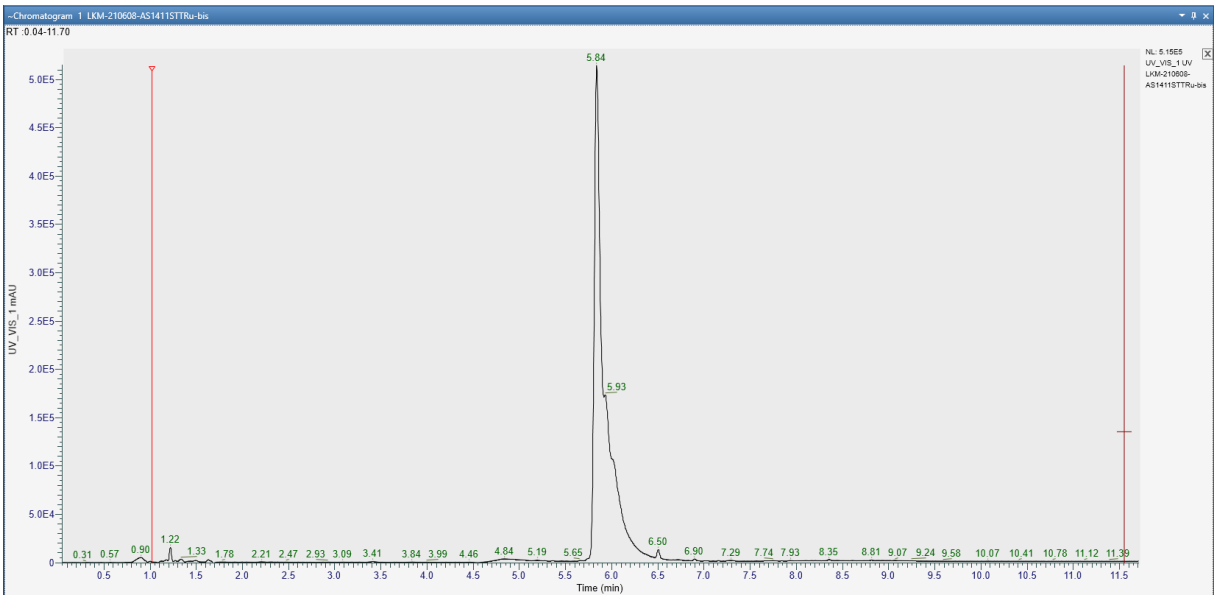
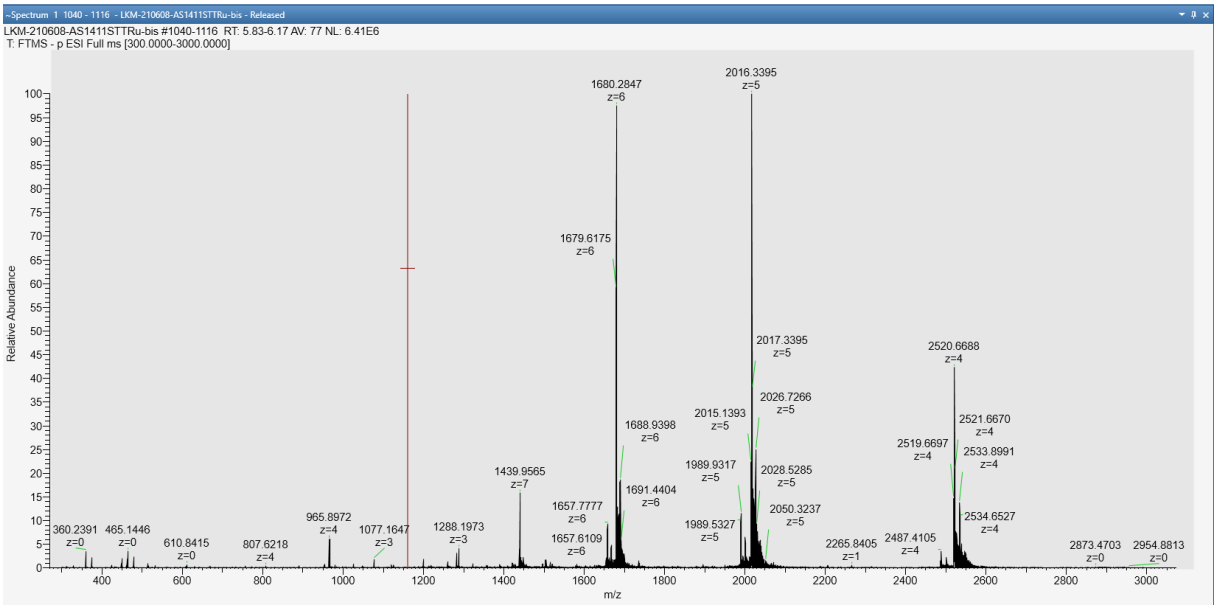


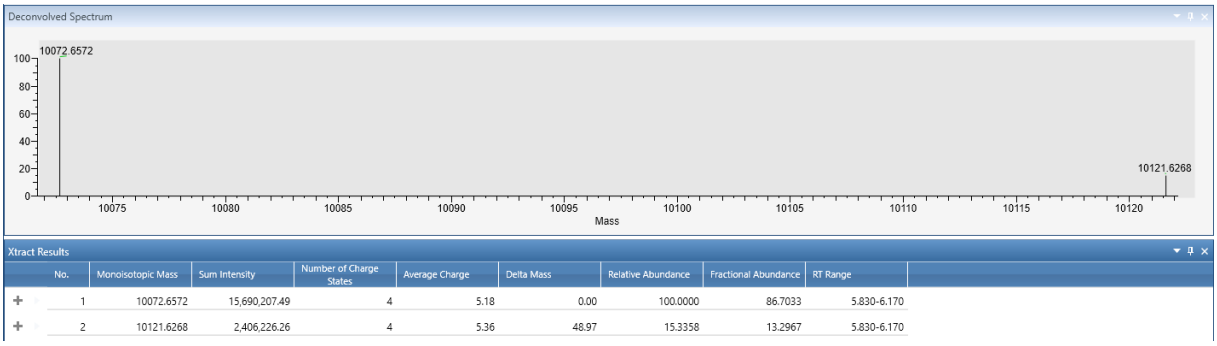
Figure S16. LCMS results for yield **AS1411-5'-TT-Ru**, **AS1411-5'-TTTTT-Ru** and **AS1411-3'-TTT-Ru**

AS1411-5'-TT-Ru

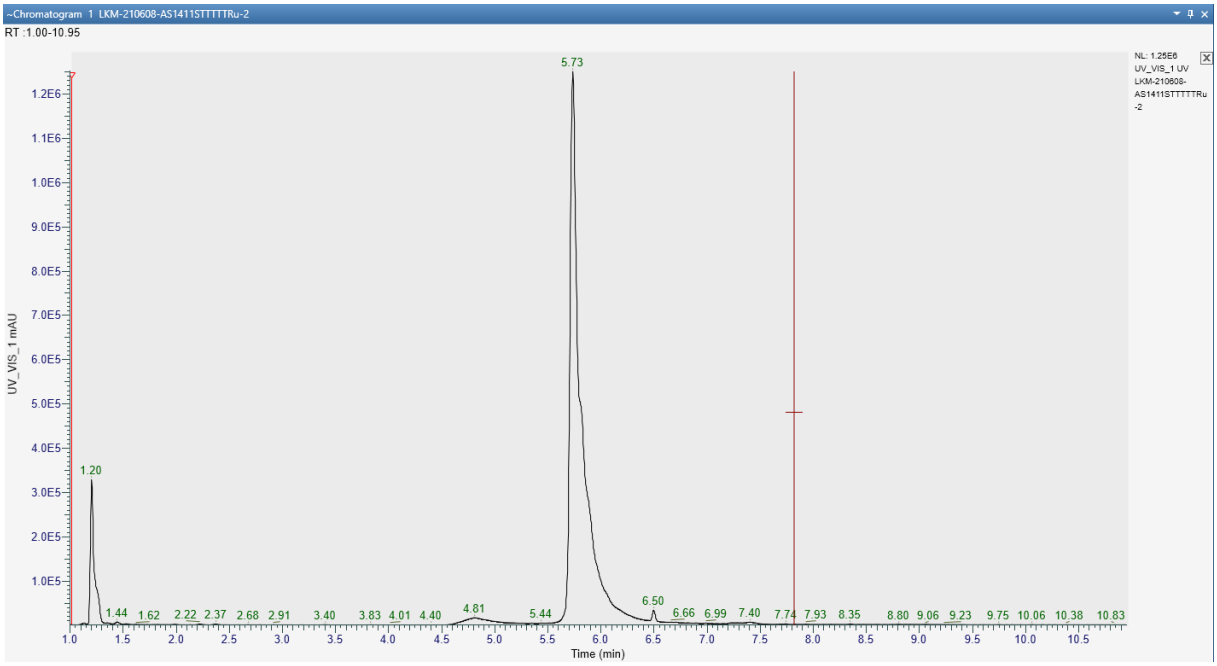


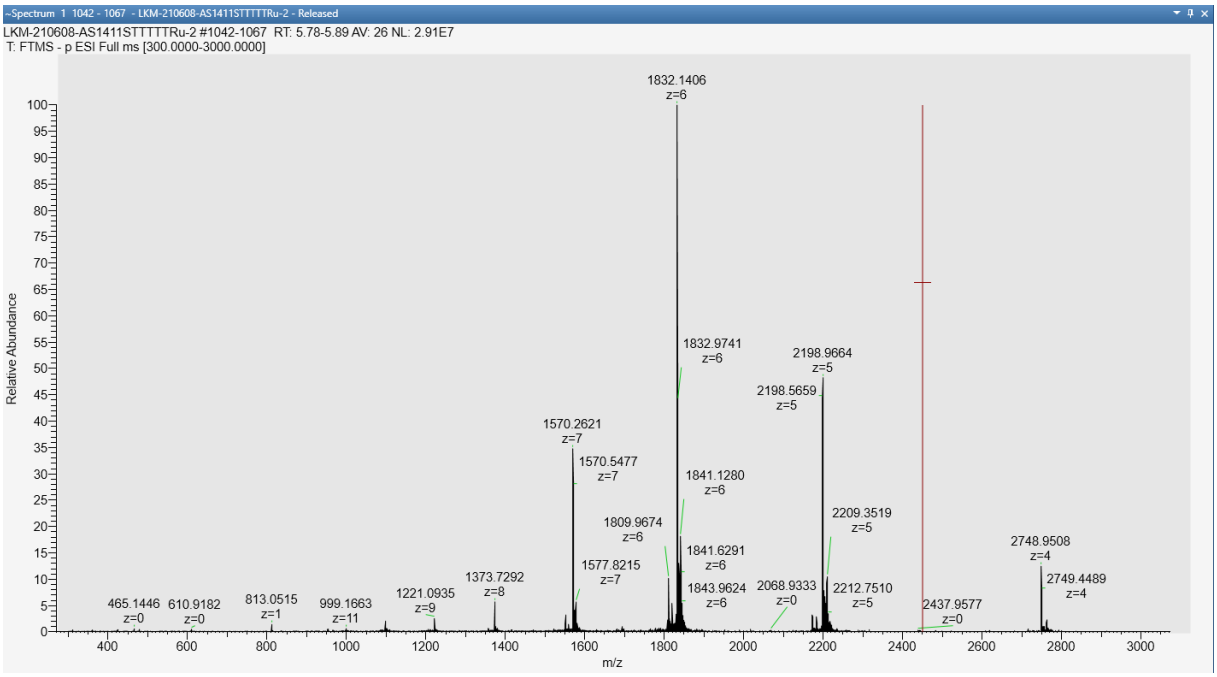


EM=10072 (tr = 5.84) (equivalent mass at tr = 5.93)

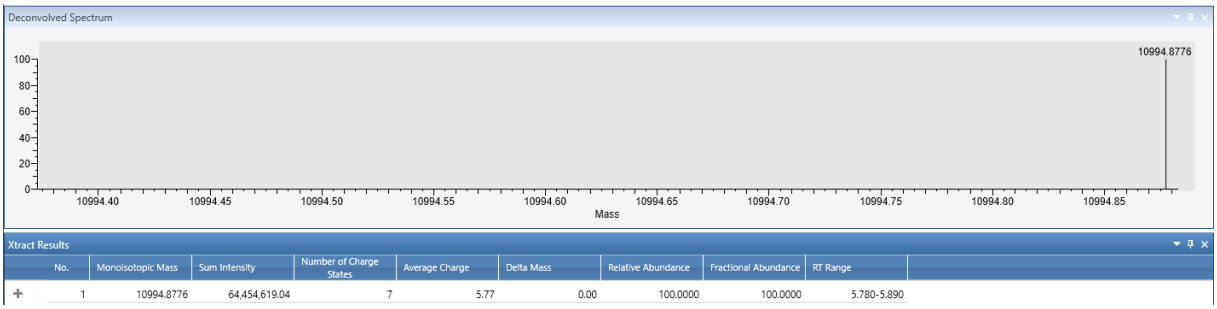


AS1411-5’-TTTTT-Ru

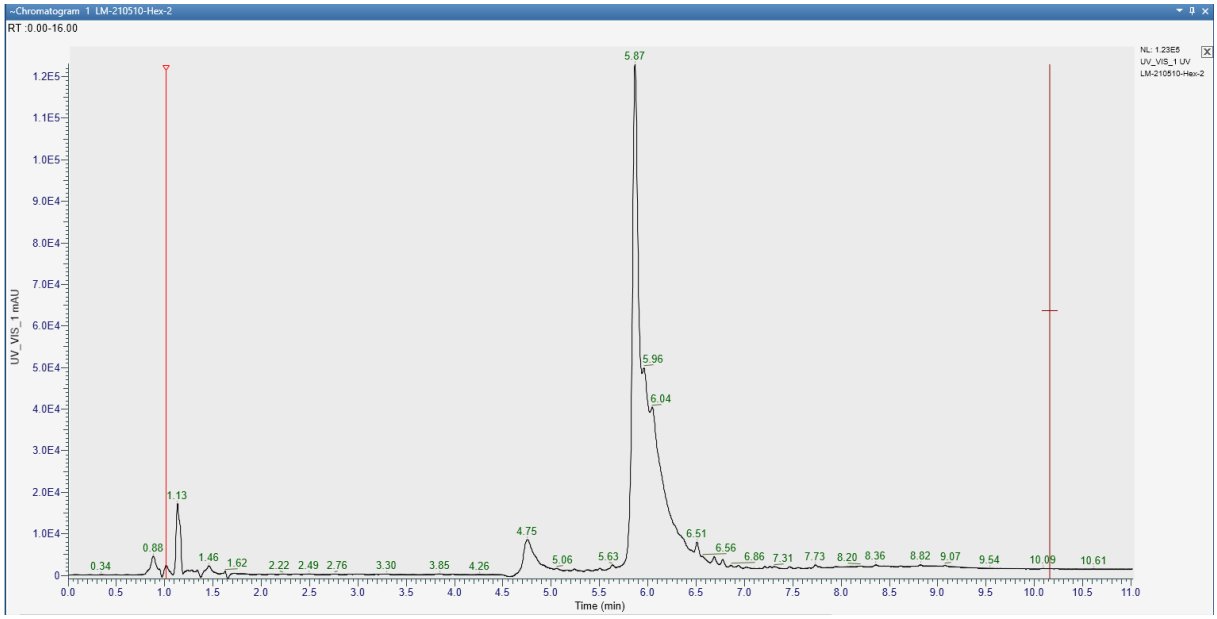


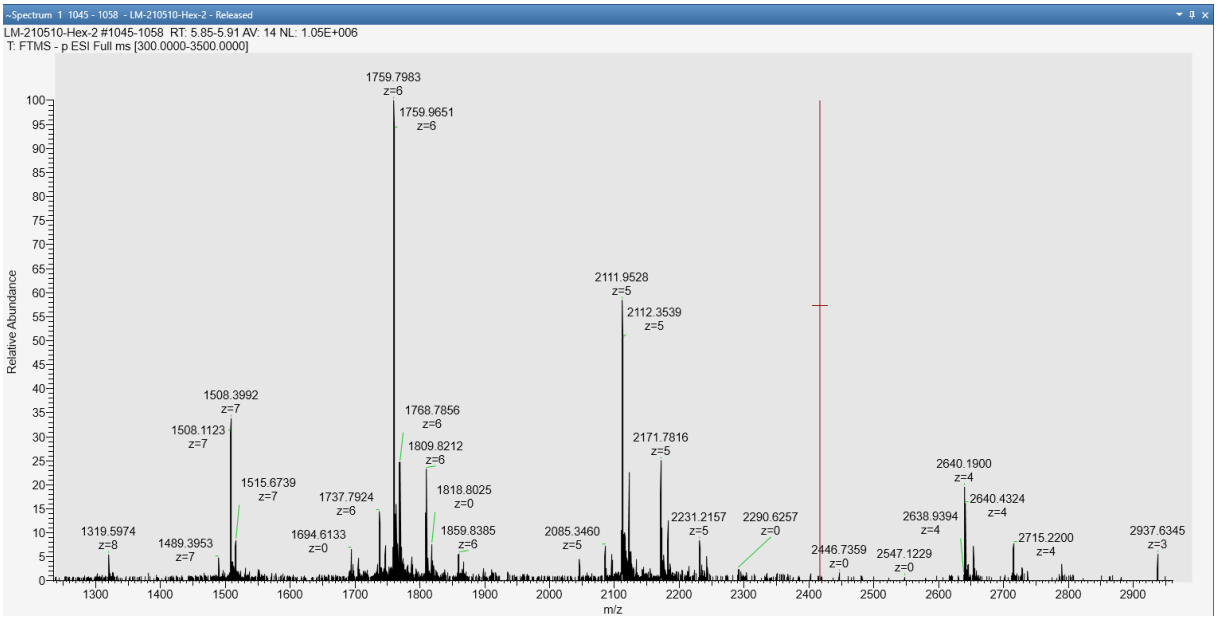


EM = 10994 (tr = 5.73)



AS1411-3’-TTT-Ru





EM = 10560 (tr = 5.87 min) (equivalent mass at tr = 5.96, tr = 6.04)

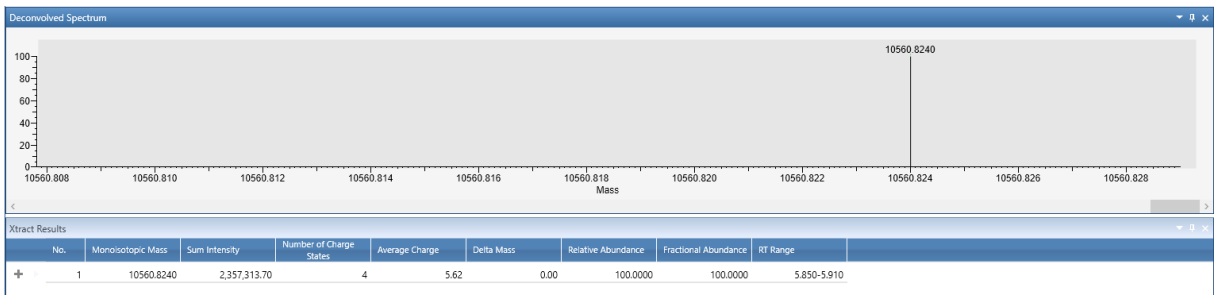


Figure S17. CD spectra of **AS1411-5'-TT-Ru**, **AS1411-5'-TTTTT-Ru** and **AS1411-3'-TTT-Ru** with increasing KCl concentration

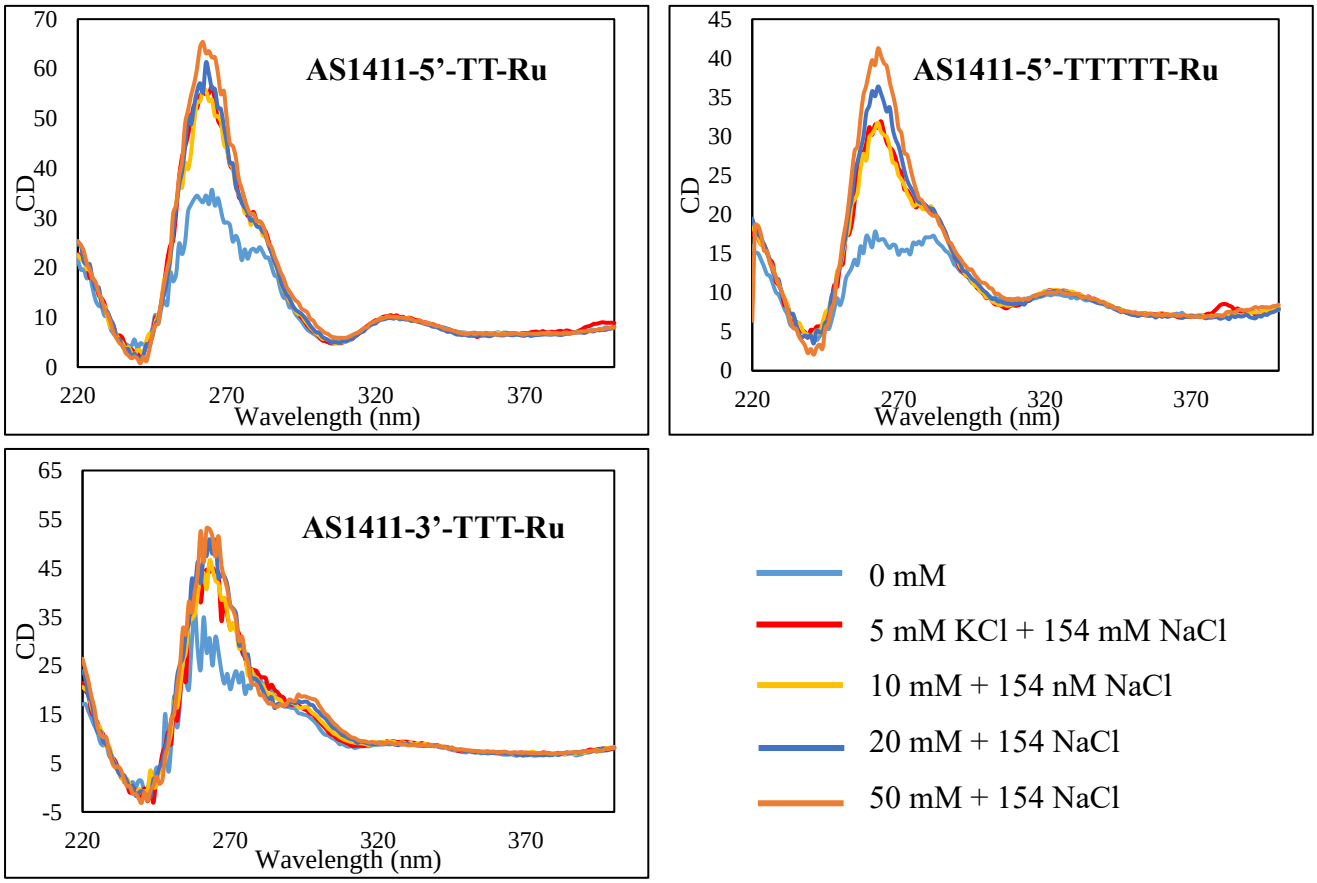


Figure S18. Thermal difference spectra of **AS1411** and **Ru-AS1411**s. Spectra normalized to maximum

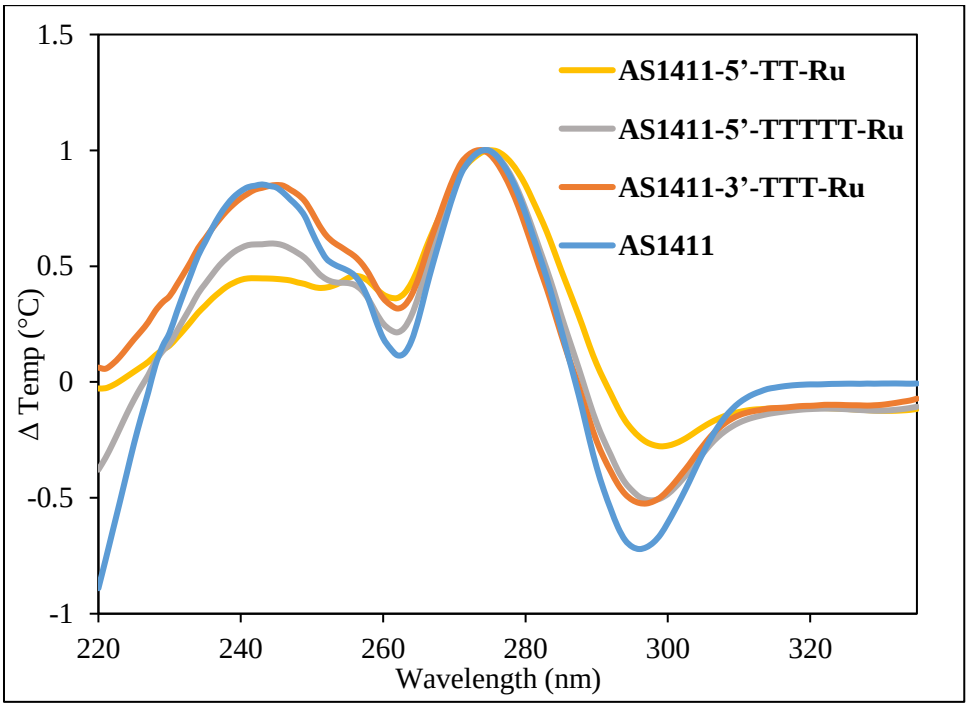


Figure S19. Confocal microscopy images of **AS1411-5'-TT-Ru** (2 μM), **AS1411-5'-TTTTT-Ru** (2 μM) and **AS1411-3'-TTT-Ru** (2 μM) in MCF-7 cells and RPE-1 cells following a two hour incubation. λ_{exc} = 405 nm λ_{em} = 600 – 750 nm scale bar = 20 microns

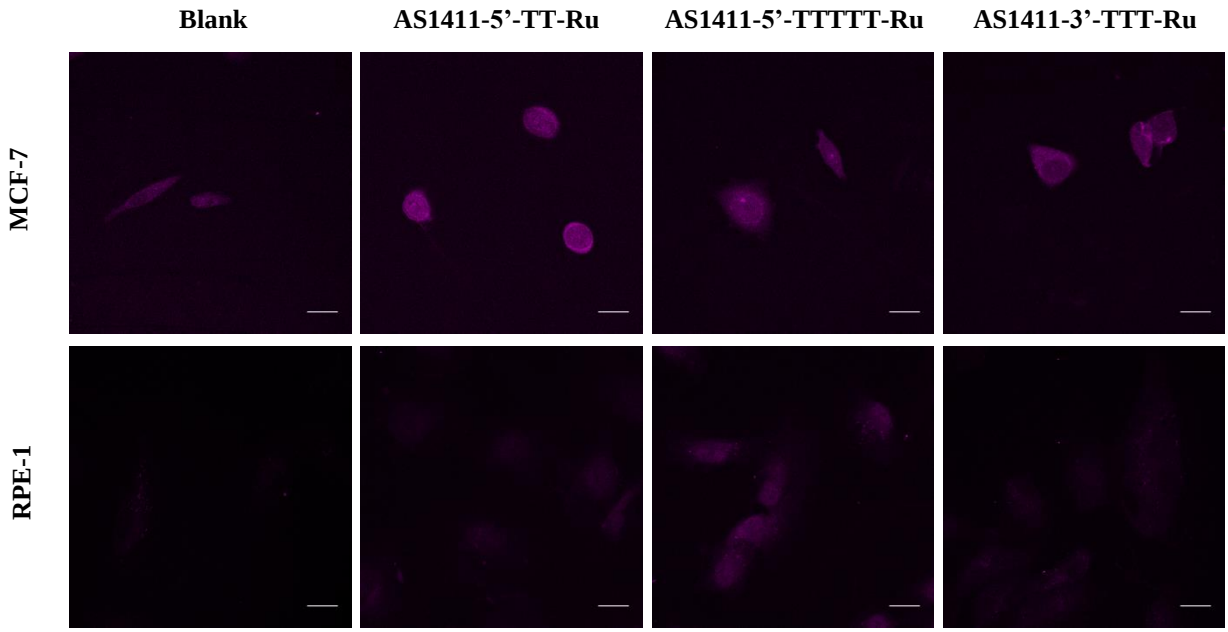
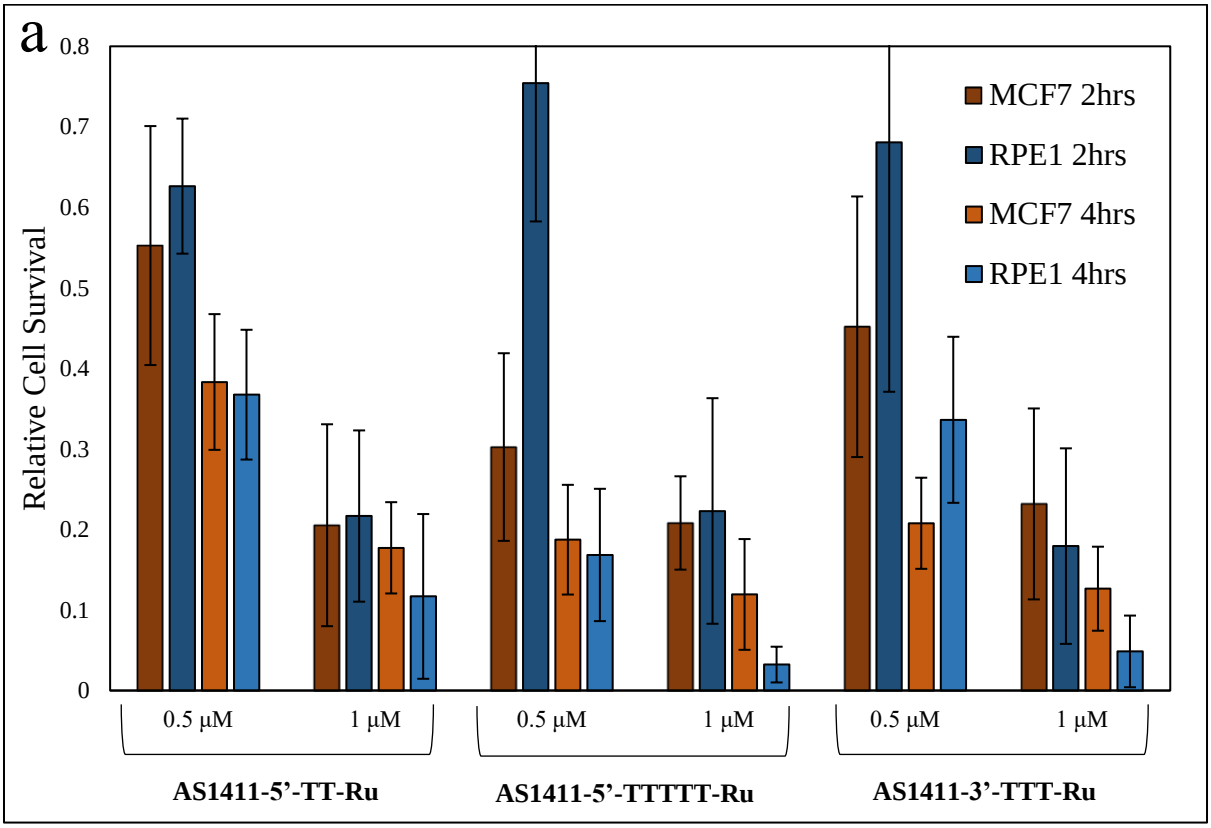


Figure S20 a) Relative cell survival following treatment with **Ru-AS1411**s and light (480 nm, 3.21 J cm^{-2}, 10 min) **b)** Relative cell survival following treatment with **Ru-AS1411s** in the dark (4 hours)



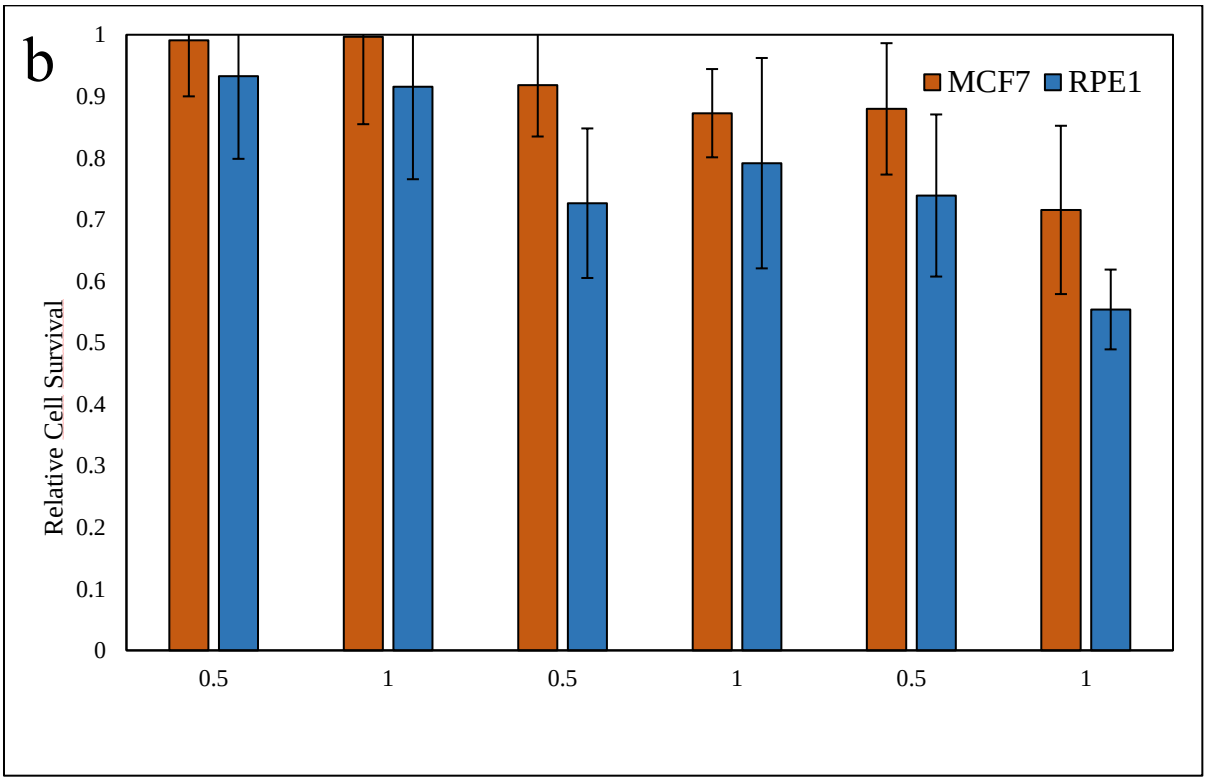


Figure S21. Media stability test. PAGE gel depicting increasing cell medium incubation times (0, 0.5, 1, 2, 4 hours and overnight). Stock samples of **AS1411** and all **AS1411Ru**s kept in 50 mM KCl.

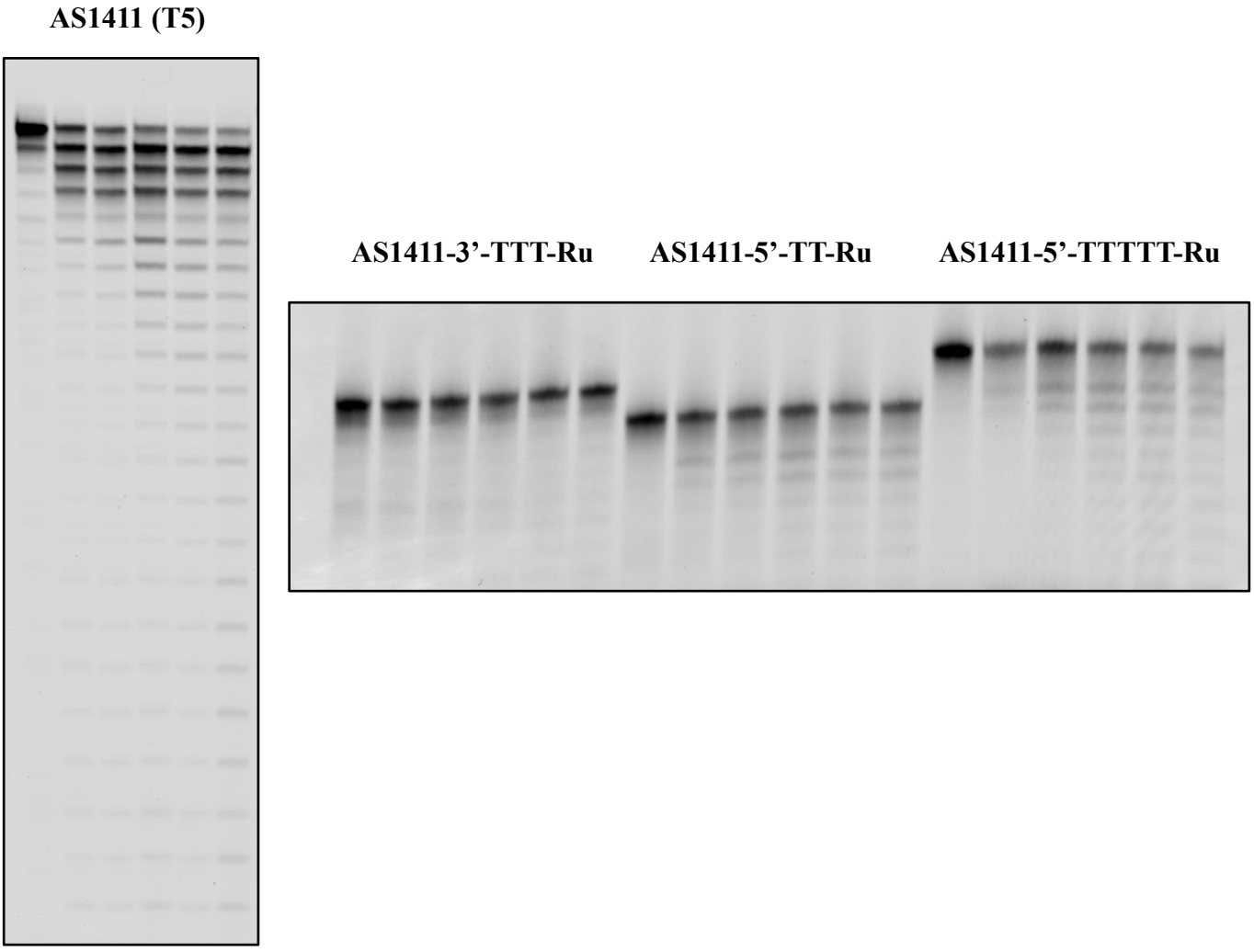


Figure S22. ^{1}H NMR spectrum of **bpyN$_3$** (400 MHz, in CD_3OD)

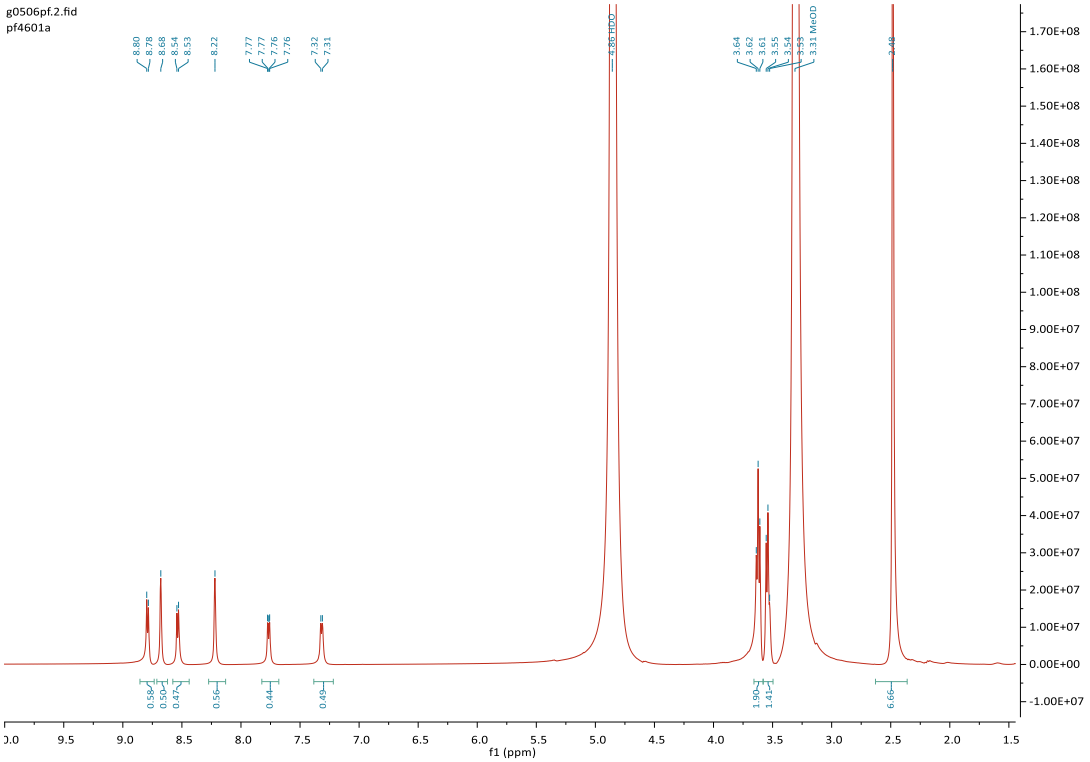
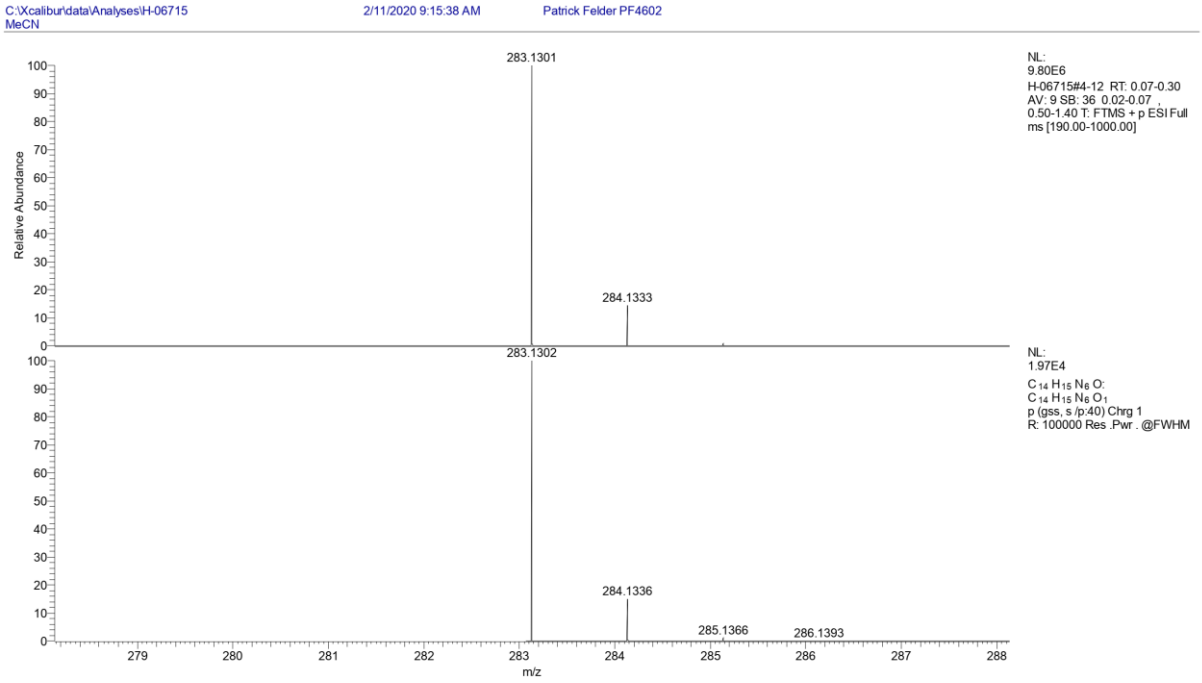


Figure S23. HR-MS ESI spectrum (positive mode) of **bpyN$_3$** (in MeCN)



Error = -0.3 ppm; Relative Intensity (%) 100

Calculation of monoisotopic masses – 1.00728 Th (-H$^+$).
–18.0338 Th (-NH4$^+$).
–22.98922 Th (-Na$^+$).
–38.96316 Th (-K$^+$).